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In this communication, we use magnetic birefringence to probe morphological changes in polymersomes during dialysis, while taking samples at regular intervals followed by the ex situ imaging of their conformations using electron microscopy. Here, we demonstrate how the morphology of polymersomes during dialysis can be probed in a continuous and non-invasive way using in situ magnetic birefringence. These measurements clearly reveal the exact times at which the morphology of the polymersomes change, making it possible to take samples for electron microscopy at the crucial points of dialysis.

Magnetic birefringence was used for in situ monitoring of the morphological changes in diamagnetic polymersomes during shape-transformation by dialysis. The birefringence was found to be very sensitive to the polymersome morphology, as determined by electron microscopy. The deflation of polymersomes into disks was observed, followed by a bending and partial inflation into stomatocytes.

Amphiphilic block-copolymers can self-assemble in water into bilayer vesicles named polymersomes. Many properties of these polymersomes, such as flexibility, permeability and functionality, can be tuned by varying the type or length of either the hydrophobic or the hydrophilic part of the block-copolymer or by adding functional groups to make them stimuli responsive. Flexibility and permeability can also be affected by the addition of an organic solvent, such as tetrahydrofuran (THF), which acts as a plasticizer for the hydrophobic part of the polymersome membrane. It has been previously demonstrated that polymersomes, self-assembled from poly(ethylene glycol)-polystyrene (PEG-PS) in a mixture of THF, 1,4-dioxane and water, undergo shape transformations into bowl-shaped structures called stomatocytes by dialysis against pure water. Also the conformation of these stomatocytes could be further manipulated by reverse dialysis against a mixture of water, THF and dioxane. This control over morphology has led to nanoparticle encapsulation by the stomatocytes and their supramolecular assembly to give stomatocyte nanorockets. These properties make polymersomes and stomatocytes very promising candidates as nanocounters in drug delivery or nanochemistry.

Until now, the effect of dialysis on the morphology of the polymersomes or stomatocytes has been investigated by taking
Further insight into the mechanism of osmotically induced shape change is presented. To explore the morphological changes we implemented a dialysis cell with a magnetic birefringence setup in a 2 T magnet (Fig. 1). Light of a HeNe laser (1.5 mW, 632.8 nm) was focussed on the upper chamber of a flow cell, which contained a PEG44–PS133 polymersome sample. Initially the sample consisted of spherical polymersomes in pure water, having a radius of 252 nm and a PDI of 0.134, as determined by dynamic light scattering (DLS). A dialysis fluid consisting of 50% water, 40% THF and 10% 1,4-dioxane was pumped through the bottom chamber at a rate of 100 mL h\(^{-1}\). The chambers were separated by a 12–14 kDa cut-off membrane. The magnetic birefringence was detected using a standard polarization modulation technique.

The birefringence was measured over a time interval of 360 minutes (blue curve in Fig. 2I). At the beginning of the experiment, the birefringence remained zero up to 170 minutes (point c), after which a rapid increase in the signal was observed. A maximum was reached at 230 minutes (point e), after which the birefringence decreased. From point g onwards, the sample showed a small birefringence, which remained constant until the end of the measurement (point h). At certain time points, magnetic field sweeps were performed where the magnetic field was reduced to zero and subsequently brought back to 2 T (indicated in Fig. 2I by the coloured lines 1–5). The field sweeps were sufficiently rapid (about one minute) to allow the subsequent birefringence signal to remain unchanged by the continuous dialysis. The birefringence always decreased to zero when the magnetic field was brought to zero. Restoring the field to 2 T also resulted in recovery of the birefringence to the same value before the field sweep, showing that the measured constant magnetic birefringence signal is not caused by drift. The inset of Fig. 2I (ESI\(^+\)) shows that the polymersomes were present with the expected spherical morphology (point a) corresponding to zero birefringence. At points b and c, no changes in conformation were observed, in agreement with the measured constant magnetic birefringence. At point d the birefringence increased to half its maximal value. The cryo-SEM images show ellipsoidal polymersomes, which can only be explained by a partial deflation of the spherical polymersomes. When the birefringence reached a maximum (point e), flat disks were observed under a cryo-SEM. Continuation of the dialysis led to the bending of the flat disks (point f) and the formation of stomatocytes (points g and h), where the structures partly inflated again. At this point, the hydrodynamic radius of the polymersomes was decreased to 218 nm with a PDI of 0.12, as determined by DLS (see ESI\(^+\)).

Measuring magnetic birefringence during dialysis offers the opportunity to stop dialysis at well-defined points to take samples for further investigation by electron microscopy. In this manner, the morphology can be related to the amplitude of the birefringence signal. Samples were taken at points a–h as indicated by the red dots in Fig. 2I (ESI\(^+\)).

Cryogenic-Scanning Electron Microscopy (cryo-SEM) images of samples (a–h) are shown in Fig. 2II. At the beginning of dialysis, polymersomes were present with the expected spherical morphology (point a) corresponding to zero birefringence. At points b and c, no changes in conformation were observed, in agreement with the measured constant magnetic birefringence. At point d the birefringence increased to half its maximal value. The cryo-SEM images show ellipsoidal polymersomes, which can only be explained by a partial deflation of the spherical polymersomes. When the birefringence reached a maximum (point e), flat disks were observed under a cryo-SEM. Continuation of the dialysis led to the bending of the flat disks (point f) and the formation of stomatocytes (points g and h), where the structures partly inflated again. At this point, the hydrodynamic radius of the polymersomes was decreased to 218 nm with a PDI of 0.12, as determined by DLS (see ESI\(^+\)).

At all points, the magnitude of the birefringence reflects the shape of the structures. All spherical polymersomes show zero...
In conclusion, we have demonstrated that magnetic birefringence can be used as a useful tool to monitor morphological changes in polymersomes resulting from dialysis in a flow cell. This method has the advantage of being non-invasive; while the morphology can be determined without disrupting the dialysis setup for sample investigation by electron microscopy. Also, because the dialysis in the flow cell is rather slow (in the order of several hours), the shape transformations can be determined at very precise points on the birefringence curve, providing samples with very specific and predictable conformations. In principle, this method could be extended to obtain more quantitative information about the rigidity of the structures in all stages of the process. However this would require a more extended theoretical analysis.

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Notes and references